OPINION

Osteopathy in complex lymphatic anomalies

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ABSTRACT

Complex Lymphatic Anomalies (CLA) are idiopathic abnormalities of the lymphatic system that also affect the soft tissues of the bones. Different CLA subtypes have varying degrees of aberrant lymphatic presentation and boney invasion. Due to these diseases' rarity and unpredictable course, the aetiology has proven to be very elusive. We gathered research on the four main CLA subtypes and discussed each one's clinical presentation, lymphatic invasion, osseous profile, and regulatory pathways connected to aberrant bone loss brought on by the lymphatic invasion in this review. We emphasise crucial proliferation and differentiation mechanisms that are shared by lymphatics and bone, as well as potential interactions between these systems that could promote lymphangiogenesis and result in bone loss.

Key Words: *Lymphatic; Bone; Lymphangiogenesis; Osteolysis; Anomaly*

INTRODUCTION

Idiopathic boney lesions brought on by aberrant lymphatic infiltration characterise Complex Lymphatic Anomalies (CLA). The lymphatic network is a one-way system that is primarily in charge of lipid absorption, immunological surveillance, and fluid balance. Interstitial fluid, a crucial component of Lymphatic Fluid (LF), is absorbed by the lymphatic system from extracellular spaces that contain a variety of proteins, small molecules, and lipids. LF is returned into the venous circulation in a single direction. The immune system, which is in charge of identifying and defending against foreign bodies, is housed within this network of vessels. The majority of tissues contain the lymphatic network, with the exception of bone, skeletal muscle, bone marrow, cartilage, and most of the eye. During foetal development, lymphatic cells primarily come from the endothelial lining of venous walls, but they can also develop from non-venous sources like mesenchymal stem cells and angioblasts. A crucial transcription factor called Prox1 is known to start LEC differentiation from blood endothelial cells .

Vascular Endothelial Growth Factor-C (VEGF-C), which emerges from veins, is the primary protein known to promote Lymphatic Endothelial Cells' (LEC's) migration and proliferation during embryogenesis. PIK3 and the mammalian Target of Rapamycin (mTOR) pathways are activated when VEGF-C binds to Vascular Endothelial Growth Factor Receptor-3 (VEGFR-3), increasing Prox1 expression and maintaining the lymphatic phenotype. Another essential signalling pathway during lymphatic growth is stimulation of the Receptor Activator Nuclear Kappa Beta (RANK) through RANK ligand (RANK-L). For lymph node organogenesis, hematopoietic Lymphoid Tissue Inducing (LTi) cells and mesenchymal Lymphoid Tissue Organiser (LTo) cells secrete RANK-L. During development, LEC are known to draw macrophages in response to RANK-L . The RANK/RANK-L signalling pathway dysfunction is known to result in aberrant lymphatic formation. In CLAs, the lymphatics in the surrounding tissues, including bone, develop and organise abnormally. In vertebrates, the skeletal system serves as a source of mineral storage and a structural support for the body . Although there is a dense network of blood and lymphatic vessels in the periosteum (connective tissue surrounding bone), lymphatic protrusion into cortical and trabecular bone is not typical.

The balance of cytokines that control the communication between bone cells and balance bone resorption and bone creation ensures that there is a constant turnover of bone.

Osteoblasts (OBs), which secrete the bone matrix, Osteocytes (OCs), which detect mechanical stresses, and Osteoclasts (OCs), which resorb the calcified bone matrix and shape the bone, work in closely regulated coordination to cause skeletal remodelling. Transcriptional factor activation For OB differentiation, runx-2 (Runt-related transcription factor-2) and osterix are necessary . The bone matrix, which is primarily made up of type 1 collagen and hydroxyapatite, is secreted by OB . In order to develop and differentiate in vitro, OB need exposure to -Glycerophosphate and ascorbic acid.

Although activating the VEGFR-3 may also stimulate OB differentiation, it is unlikely that VEGF-C would do so on its own . Continuous matrix secretion causes OB to become enmeshed in the bone, where they later undergo osteocyte differentiation .

In response to diverse stimuli, osteoocytes secrete RANK-L, osteopontin, Macrophage Colony-Stimulating Factor (M-CSF), and sclerostin, which are important regulators of OB and OC. When exposed to M-CSF released by OB and/or osteocytes, Hematopoietic Stem Cells (HSCs) develop into macrophages (pre-osteoclasts). By being exposed to RANK-L, which is secreted by OB and/or osteocytes, these macrophages are further differentiated into mature osteoclasts to

become mature and active OCs . Tumour Necrosis Factor-alpha (TNF-alpha), Interleukin-1 beta (IL-1 beta), Interleukin-6 (IL-6), and other inflammatory cytokines are known to impair OB differentiation and function while promoting OC maturation . Through VEGF-C, OC can also stimulate themselves. Accelerated differentiation and function occur in OC as a result of VEGF-C being secreted and its primary receptor VEGFR-3 being activated as a result of RANK-L stimulation.

Complex Lymphatic Anomalies (CLAs)

Gorham-Stout disease (GSD), General Lymphatic Anomaly (GLA), Kaposiform Lymphangiomatosis (KLA), and Central Conducting Lymphatic Anomaly (CCLA) are the four illnesses that make up the CLAs and have both overlapping and different clinical symptoms . Due to their rarity and erratic course, the aetiology of these diseases has proven to be very elusive over time. CLAs are thought to be developmental in nature, with no known sex preference, and primarily affecting those under the age of 40. According to the International Society for the Study of Vascular Anomalies (ISSVA) , each CLA has a distinct lymphatic phenotype. CLAs have been linked to somatic activating mutations in the PIK3CA-Akt, Rat sarcoma virus, and Mitogen-Activated Kinase (RAS-MEK), as well as PIK3CA, Catalytic Subunit Alpha, and Protein Kinase B (PIK3CA-Akt), genes . A deeper comprehension of the pathology of CLAs will aid in the creation of novel diagnostics and evidence-based therapeutics to treat soft tissue and skeletal problems in patients who have been successfully identified. Here, we summarise the most recent information on each CLA's clinical manifestation, lymphatic phenotype, and bone phenotype before presenting potential regulators during bone loss.

1. Gorham Stout Disease: The exceedingly rare disorder known as "vanishing bone disease," or GSD, is characterised by idiopathic and progressive osteolysis that affects one or more bones. The occurrence of both cortical and trabecular bone loss brought on by the gradual invasion of lymphatics into bone is what distinguishes GSD from other conditions. GSD individuals exhibit both cortical and trabecular osteolysis, in contrast to other CLAs who only experience trabecular bone loss. Typically, GSD only affects one or a few bones locally. The structural location and disease course of GSD dictate its functional effects. Bone discomfort, inflammation, restricted movement, skeletal deformity, and chylothorax are among the main symptoms of GSD patients. Chylothorax, or the buildup of lymphatic fluid in the thoracic cavity, is a potentially fatal side effect experienced by all CLAs. In GSD compared to other CLAs, skull involvement and fractures are more common. Recently, it has been demonstrated that lymphatic malformations are brought on by hyperactivation of the RAS-MEK pathway, which is caused by a gain-of-function somatic mutation in KRAS (p.G12V) of GSD LECs . Several cancer types, including breast, colorectal, and liver cancers, as well as abnormal lymphangiogenesis are frequently linked to this pathway. Most recently, it was discovered that a mouse model with the same activating somatic KRAS mutation (p.G12D) replicated aberrant lymphatics and bone invasion reported in GSD patients.

2. General lymphatic anomaly: Dilated lymphatic channels and widespread lymphatic invasion, which affect a number of soft tissues and bone, are the hallmarks of GLA . A generalised distribution with multiorgan dysfunction and lytic trabecular boney lesions distinguishes GLA from GSD. The ribs and spine are particularly affected by GLA, which frequently affects the axial skeleton. The range of symptoms that vary from case to case make it challenging to diagnose due to this variable presentation affecting many places in the body. GLA can happen to anyone at any age, however symptoms frequently appear more frequently in adolescents and teenagers before the age of 20.

3. Kaposiform lymphangiomatosis: KLA is a highly aggressive variety of GLA that only manifests as aberrant lymphatic channels filled with spindle endothelial cells . KLA exhibits an ongoing engagement with significant morbidity and a high mortality rate. The 5-year survival rate was found to be 51%, with an overall survival rate of 34% in a retrospective investigation of 20 patients between 1995 and 2011; the most common cause of death was cardiorespiratory failure brought on by consumptive coagulopathy. Young patients with this extremely aggressive subtype may manifest with organ failure, pleural and pericardial effusions, ascites, discomfort, and boney osteolysis.

Similar to GLA, the thoracic cavity is the primary location of the afflicted bones in KLA. A distinguishing characteristic of KLA is its capacity to damage various organs with pleural effusions, ultimately leading to respiratory discomfort in the thoracic cavity. KLA and GLA patients appear with comparable boney phenotypes. Lymphatic channels with concentrated regions of spindle-shaped "kaposiform" endothelial cells that are positive for Podoplanin and PROX-1 (markers for lymphatic tissue) are indicative of a KLA diagnosis .

Numerous characteristics of GLA are present in KLA as well, including non-progressive lesions in the medullary cavity of the bone, widespread lymphangiomatosis, and multifocal lymphatic malformations affecting the viscera, thoracic, and abdominal cavities.

KLA is brought on by mutations in the NRAS and CBL proto-oncogene genes, which were found in patient tissue biopsies. The adoption of noninvasive diagnostics for KLA is necessary since tissue biopsy may require surgical intervention close to important organs, which could cause serious problems aggravating the condition. A valid biomarker for KLA was shown to be elevated Angiopoietin-2 (Ang-2)—a protein involved in blood vessel formation and stability. Additionally, after successful treatment, Ang-2 levels return to normal, suggesting that this marker may be used to monitor disease development. Interestingly, vessel maturation and stability have been reported to be negatively impacted by Ang-2 . Despite these findings, more investigation is required to establish Ang-20's role in KLA pathogenesis.

Lymphatic bone invasion

It has been established that aberrant lymphatic phenotypes found in CLAs are caused by somatic activating mutations in the PIK3CA or RAS/MEK pathways.

A mouse model that overexpresses the activating PIK3CA mutation in cells expressing Prox1 (a LEC marker) was created in order to mimic

the phenotype that these mutations in people induce. This model specifically expressed the mutation that causes lymphatic tissue to hyperproliferate and malfunction, just like in GLA . Sirolimus (Rapamycin), a medication successfully used in some individuals with CLAs , was shown to both prevent and decrease lymphatic abnormalities in mice. Mice overexpressing VEGF-C in osterix-expressing cells (early OB marker) were discovered to drive lymphatic invasion from peripheral lymphatics across muscle, cortical bone, and into the bone medullary cavity in order to assess lymphatic invasion into bone. This model demonstrated how bone can be invaded and colonised by peripheral lymphatics. The hypothesis that aberrant lymphatics develop from pre-existing lymphatic vessels is supported by this model, despite the fact that it may not directly depict a CLA. This study adds to the body of evidence that LEC alone in bone, with or without mutation, causes abnormal bone loss, whereas somatic mutations may cause LEC to migrate into bone.

Current Knowledge of CLA Osteopathy
Despite the most recent discovery of somatic mutations in each illness, it is still unclear how CLA causes bone loss. A somatic genetic mutation in bone cells has not yet been connected to the bone loss observed in CLA. A biopsy sample from a patient with GSD revealed mutations in the genes for TNF Receptor Superfamily Member 11a (TNFRS11) and Triggering Receptor Expressed in Myeloid Cells 2 (TREM2). Through accelerated OC differentiation and excessive osteolysis, these mutations are known to cause bone loss .

We cannot tell whether these mutations are linked to CLAs or which cell type is involved as these mutations were only reported in one GSD patient. There is no aberrant skeletal phenotype prior to the onset of dysfunctional lymphatics. This makes us think that the cause of bone loss is the direct interaction between lymphatics and bone. Non-pathogenic lymphatic cell lines were intra-tibially injected into mice to investigate the impact lymphatic invasion had on bone . In mice, this method caused severe osteolysis, which resulted in full bone loss within weeks.

Furthermore, this effect was closely related to OC activation via LEC-mediated M-CSF secretion. Various CLAs have reported increased OC activity , whereas GSD have reported little to no bone formation to replace lost bone. There aren't many cases of CLA patients' bone progenitor cells being successfully identified and culture-differentiated into OC or OB. Initial reports on GSD patient biopsy revealed marked osteoclastic resorption in both trabecular and cortical bone . Through histological analysis, this group performed six different biopsies on each patient, and all of them showed evidence of significant osteoclast-mediated bone resorption. When compared to healthy controls, cultured Peripheral Mononuclear Cells (PMCs) from GSD patients increased OC differentiation and function. A recent study used many patients and repeated the isolation strategy with comparable outcomes. The anatomical variations between the patient and control OC are mentioned in both studies. These patients' OC had a more spindle-shaped appearance, which indicated that the patients' cells had a higher rate of motility, which increased the resorption activity of the OC. GSD OC microarray research uncovered dysregulated genes brought on by the condition. Low-density Lipoprotein Receptor-Related Protein 6 (LRP6), a crucial element in OC maturation , was one of these. It was elevated in GSD OC. OC are frequently hyperactive in GSD patients, however this is not a common observation in all GSD patients observed. This discrepancy may be caused by patient samples

with variable disease activity or progression. High OC activity would be anticipated in patients who have seen rapid bone loss. Despite conflicting data, it is thought that hyperactive OC are the primary initiators of the osteolysis mechanism seen in CLA patients. Reduced OB mineral deposition has been linked to slower fracture healing, according to reports from GSD patients . Rossi et al. recovered PMCs and Mesenchymal Stem Cells (MSC) from GSD patients and successfully differentiated them into OC and OB, respectively, in recent years .

Proposed CLA osteopathy
However, both bone and lymphatics readily secrete various cytokines and growth factors that cause lymphangiogenesis and bone resorption (Figure 4). Under normal circumstances, lymphatic and bone cells do not interact. We hypothesise that somatic mutations are mostly responsible for lymphatic invasion towards bone because normal lymphatics can cause aberrant bone loss .Upon entering the bone, lymphatics directly interact with bone cells, causing different types of osteolysis, as seen in CLAs.

VEGF-C exposure is a major driver of lymphangiogenesis. When triggered by RANK-L, OC produce VEGF-C in a way that is SRC Proto-Oncogene, Non-Receptor Tyrosine Kinase (Src) phosphorylation-dependent. Furthermore, VEGF-C therapy is known to stimulate OC similarly to M-CSF. Additionally known for secreting VEGF-C are MSC and macrophages (OC progenitors) .

These VEGF-C sources might easily promote additional lymphatic infiltration and start bone resorption. During lymphatic invasion and osteolysis, RANK-L and M-CSF activation of both lymphatic and OC may be crucial. The growth of lymphatic nodes inside the lymphatic system requires RANK/RANK-L activation. It was demonstrated that RANK-L is required for both OC differentiation and function and lymphatic development by the failure of mice lacking either RANK-L or its receptor to form lymph nodes . Local macrophage aggregation is induced by RANK-L activation of LEC and may promote osteoclast fusion . In vitro, LEC release M-CSF and RANK-L similarly to OB and osteocytes .

Then, lymphatic vessels carry RANK-L and IL-6 (stimulants of OC differentiation)-secreting immune cells like helper T-cells.

Osteocyte osteolysis in GSD
The distinctions between the bone involvement of GSD and other CLAs have not yet been determined. GSD has sparked the most attention to clarify its osteopathy because of its regionally aggressive cortical and trabecular bone loss. Higher concentrations of IL-6, Vascular Endothelial Growth Factor-A (VEGF-A), Cross-Linked Telopeptide Of Type 1 Collagen (ICTP), and sclerostin were found in the serum of six GSD patients, according to an analysis . Through the secretion of RANK-L by OB and osteocytes, the cytokine IL-6, which is associated with inflammation, is known to promote bone resorption. A well-known promoter of blood vessel angiogenesis, VEGF-A is probably related to the expansion of angiomatous lesions in CLA patients. Sclerostin is a protein frequently released by osteocytes that binds to and inhibits OB, whereas ICTP is a sign for OC activity . These blood indicators linked to GSD explain the severe osteolytic phenotype and contribute to overall bone loss in individuals. Localised lymphatic invasion brought on by somatic mutations can be linked to regional involvement in GSD. Despite this, cortical involvement is still a remarkable aspect of GSD that needs further study. GSD is the only

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CLA that reports having high sclerostin levels. Sclerostin osteocyte activation is known to cause osteocytic osteolysis, where osteocytes resorb surrounding bone (perilacunar remodelling), in addition to OB inhibition. During hyperthyroidism, osteoporosis, tumours, and calcium deficiency, osteocytic osteolysis has been found to reduce bone mineralization with increased bone resorption and reduced bone healing. When compared to control patients, a histological analysis of GSD patients revealed enlarged lacunae .